

Molecular and bioacoustic divergence in *Mantidactylus granulatus* and *M. zavona* n.sp. (Anura: Mantellidae): bearings for the biogeography of northern Madagascar

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We analysed the genetic and bioacoustic variability in *Mantidactylus granulatus*, a semi-arboreal frog species endemic to the northern portion of Madagascar. Advertisement calls had a longer note duration in northeastern as compared to northwestern populations. Specimens from mid-altitudes in the Tsaratanana Massif (Northern Central Region) showed distinct call differences. They also had a high sequence differentiation in a fragment of the mitochondrial 16S rRNA gene (pairwise divergence 8.6 %), while all other populations were genetically rather uniform. This Tsaratanana population is described as the new species *Mantidactylus zavona*; it is also morphologically distinct from the sympatric *M. granulatus* in that it has a smaller body and a shorter, wider head. The relatively small differences between *M. granulatus* from different sites in northern Madagascar (pairwise 16S divergences up to 1.4 %), including the isolated Montagne d'Ambre, supports a biogeographic scenario in which the lowland rainforests in this area were connected in Pliocene–Pleistocene times. By contrast, the presence of endemic species at higher altitudes of the Tsaratanana Massif may be seen as evidence for a longer isolation of the habitats at these elevational levels.

Key words: Amphibia, *Mantidactylus granulatus*, *M. zavona* n.sp., advertisement calls, 16S rRNA.

INTRODUCTION

In the speciose anuran genus *Mantidactylus* Boulenger, 1895, some lineages have their centre of diversity in the northern portion of Madagascar. One of these is *Phylacomantis* Glaw & Vences, 1994, which is one of the currently 12 subgenera in *Mantidactylus* (Dubois 1992; Glaw & Vences 1994 2001). These frogs are in great part characterized by their probably specialized reproductive modes. Exceptions in the subgenus *Phylacomantis* are the species of the *M. pseudoasper* group (*Phylacomantis*) which have free-swimming tadpoles. Other *Phylacomantis* (all included in the *M. granulatus* group) generally call along brooks, but their larval stages have never been collected. They may undergo development in hidden nests as non-feeding tadpoles. This hypothesis has been proposed by Glaw & Vences (1994) for *M. granulatus* (Boettger, 1881), a species that is known from

several regions in northern and western Madagascar (Blommers-Schlösser & Blanc 1991).

The northern part of Madagascar comprises four biogeographic regions according to Angel (1942), Glaw & Vences (1994) and Raxworthy & Nussbaum (1995): the North, Northeast, Northwest (Sambirano) and Northern Central regions. These regions largely correspond to the rainforest blocks of Montagne d'Ambre (North), Marojejy, Ambolokopatrika-Betaolana, Anjanaharibe-Sud and Masoala (northeast), Tsaratanana (Northern Central) and Manongarivo (Northwest). Montagne d'Ambre is currently isolated from the other forest parcels, and indeed represents a true forest island within the surrounding xeric habitat in northern Madagascar. On the other hand, the forest complex constituted by Anjanaharibe-Sud, Ambolokopatrika-Betaolana, Marojejy and Masoala in the eastern part and Manongarivo-Tsaratanana in the western part is likely connected. The current

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Table 1. Temporal and spectral characteristics of advertisement calls of *Manitadactylus granulatus* and *M. zavona*.

| | Antsahamanara (Tsaratana) | Nosy Be | Andampy (Tsaratana) | Andapa | Andrakata |
|------------------------------|--|--|--|--|----------------------|
| Species | <i>M. zavona</i> | <i>M. granulatus</i> | <i>M. granulatus</i> | <i>M. granulatus</i> | <i>M. granulatus</i> |
| Biogeographic region | Northern Centre | Northwest | Northern Centre | Northeast | Northeast |
| Recording date | 3 February 2001 | 22 January 1992 (18:30) | 10 February 2001 | 4 March 1995 (21:20) | 21 March 1991 |
| Recording temperature | 24 °C | 26 °C | 25 °C | 24 °C | 22 °C |
| Notes per note series | 3 (1–6) | 1 (2) | 1 | 1 | 1 |
| Note duration | 58–88 ms (73 ± 9 ms, <i>n</i> = 11) | 109–137 ms (125 ± 8 ms, <i>n</i> = 16) | 135–174 ms (156 ± 13, <i>n</i> = 10) | 190–250 ms (223 ± 16 ms, <i>n</i> = 55) | c. 230 ms |
| Inter-note interval duration | 98–124 ms (114 ± 8 ms) | 324–504 ms (374 ± 46 ms, <i>n</i> = 15) | 705–1341 ms (969 ± 214 ms, <i>n</i> = 10) | 833–1395 ms (1140 ± 216 ms, <i>n</i> = 8) | – |
| Note repetition rate | 6.2/s | 2.1/s | 0.8/s | 0.7/s | c. 1/s |
| Frequency | 1200–5300 Hz | 1500–4000 | 2700–3900 Hz | 1250–3300 Hz | 1500–3500 Hz |
| Dominant frequency | 2800–4000 Hz | – | 3000–3550 Hz | 2300–3300 Hz | – |

extension of these forest corridors is largely unknown, and it is more realistically represented by a patchwork of forest parcels (Anon. 1999).

During recent fieldwork we noted differences in advertisement calls between populations attributed to *M. granulatus*, one of the few frog species known from all four biogeographic regions in northern Madagascar. In the present paper, we use *M. granulatus* as a model organism to test for inter-regional differences within wide-ranging species. We present bioacoustic and molecular data from populations in the four regions to assess whether they are actually conspecific or may constitute unrecognized cryptic regional endemics, and consequently describe one new species identified by these means.

MATERIALS & METHODS

Frogs were collected during the night, with the aid of electric torches, mainly by localizing calling males. They were sacrificed using chlorobutanol, fixed in 5 % formalin or 90 % ethanol, and preserved in 70 % ethanol. Specimens studied in this paper are stored in the collections of the Muséum National d'Histoire Naturelle, Paris (MNHN); Museo Regionale di Scienze Naturali, Torino (MRSN); Parc Botanique et Zoologique de Tsimbazaza, Antananarivo (PBZT), Université d'Antananarivo; Senckenberg Museum, Frankfurt (SMF); Département de Biologie Animale (UADBA); Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn (ZFMK); and Zoologische Staatssammlung München (ZSM). For voucher numbers, see Appendix 1 and Table 1. For some still uncatalogued material in the UADBA collections we give the field numbers of M. Vences (UADBA-MV), and for material in the MRSN collection we also give the field numbers of F. Andreone (FAZC).

Specimens were collected at various sites on the Tsaratana Massif: (1) Andampy, located in the Manarikoba Forest, 14°02'32"S, 48°45'42"E, 730 m altitude; (2) Antsahamanara, also located in the Manarikoba Forest, 14°02'42"S, 48°47'04"E; c. 1100 m altitude; (3) an unnamed site in the Manarikoba Forest at 14°02'24"S, 48°47'02"E which we simply refer to as 'Manarikoba'; (4) an additional site not in the Manarikoba Forest,

namely Andranomamelona (850 m altitude).

Morphological measurements were taken by M.V. using a calliper, to the nearest 0.1 mm: SVL (snout–vent length), HW (head width), HL (head length), ED (horizontal eye diameter), END (eye–nostril distance), NSD (nostril–snout tip distance), NND (nostril–nostril distance), TD (horizontal tympanum diameter), HAL (hand length), FORL (forelimb length), HIL (hind-limb length), FOL (foot length), FOTL (foot length including tarsus), IMTL, IMTH (length and height of inner metatarsal tubercle).

Advertisement calls were recorded with tape recorders using external microphones. They were analysed either with a MEDAV sound analysing system using the software Spekro 3.2, or on a PC using the software Cooledit (Syntrillium Corp.). Temporal and metric measurements are given as range, with mean \pm standard deviation and number of measured units in brackets.

A fragment of the mitochondrial 16S rRNA gene was amplified and sequenced using primers and protocols of Vences *et al.* (2000). Outgroup choice was based on a comprehensive phylogenetic study of the subgenera *Phylacomantis*, *Laurentomantis*, and *Gephyromantis* (Vences & Glaw 2001). In the molecular trees obtained in the latter work, a clade containing *Mantidactylus granulatus* and *M. zavona* was supported by high bootstrap values. It was the sister group of a second lineage containing *M. cornutus* Glaw & Vences, 1992, *M. leucomaculatus* (Guibé, 1975), *M. tandroka* Glaw & Vences, 2001, and *M. tschenki* Glaw & Vences, 2001. We chose *M. leucomaculatus* as the outgroup in the present study because of its molecular affinities to *M. granulatus* (Vences & Glaw 2001), and its similar morphology (paired blackish vocal sac and absence of interocular tubercles and of distinct dorsal ridges).

The sequenced fragments of the 16S rRNA gene had lengths of up to 557 nucleotides (nt). However, in the outgroup a large part of the fragment could not be reliably sequenced, probably because of the inadequate preservation of the tissue of this specimen. We therefore restricted our analyses to a section of 287 nt which was common to all obtained sequences. Alignment of the restricted sequence section was possible by inclusion of gaps at three positions, corresponding to one insertion and one deletion in the outgroup, and one insertion in all *M. granulatus* (lacking in *M. zavona*).

Sequences were deposited in public databases; EMBL/Genbank accession numbers are as follows.

M. granulatus: Andampy (ZSM 645/2001, AJ314794); Andapa (ZFMK 59963, AF215325); Montagne d'Ambre (UADBA-FG/MV 2000.362; AJ315926); Nosy Be (juvenile, not preserved; AJ315927). *M. zavona*: Manarikoba (UADBA 10020; AJ315925); Antsahamanara (ZSM 627/2001, AJ314795). *M. leucomaculatus*: Marojejy (ZFMK 59953, AJ314805).

Phylogenetic analyses were carried out using PAUP*, version 4b8 (Swofford 2001). Since no general agreement exist in the literature about how to treat insertions and deletions in phylogenetic analysis of ribosomal RNA gene sequences, we performed three separate maximum parsimony searches: (1) coding gaps as fifth character state in an all-evidence approach, (2) coding gaps as uncertainties, and (3) excluding all gapped sites from the analysis. We also carried out neighbour-joining searches using LogDet distances which are robust against possible variation of sequence evolution among lineages (Lockhart *et al.* 1994). Additional analyses were performed using the complete gene fragment, entering lacking sections in some sequences as missing data.

RESULTS

Bioacoustic differentiation in *Mantidactylus granulatus*

Call recordings were available from five localities. At four localities, calls were emitted at night as series of regularly repeated single notes (Fig. 1): Nosy Be (type locality), Andampy, Andapa and Andrakata. Notes were composed of 31–39 pulses (35 ± 3 , $n = 10$) as ascertained in calls from Andampy. Table 1 gives temporal and spectral characteristics of these calls.

By contrast, at Antsahamanara, night calls were emitted as groups of several (2–6) rapidly repeated notes (Fig. 1), although single notes were sporadically also heard. Most frequent were three-note calls; these lasted 438–523 ms (486 ± 35 ms, $n = 4$), interval duration between calls was 1033–1188 ms (1117 ± 49 ms, $n = 3$). Notes were narrowly pulsed, with no distinct pulses discernible on the oscillogram. Within one note, intensity decreased gradually. Temporal and spectral characteristics of the notes are given in Table 1.

In general structure, these calls were similar to diurnal calls from Nosy Be which were described and assigned to *M. granulatus* by Glaw & Vences (1994). The identity of these calls, however, remains uncertain as the calling specimens were not collected.

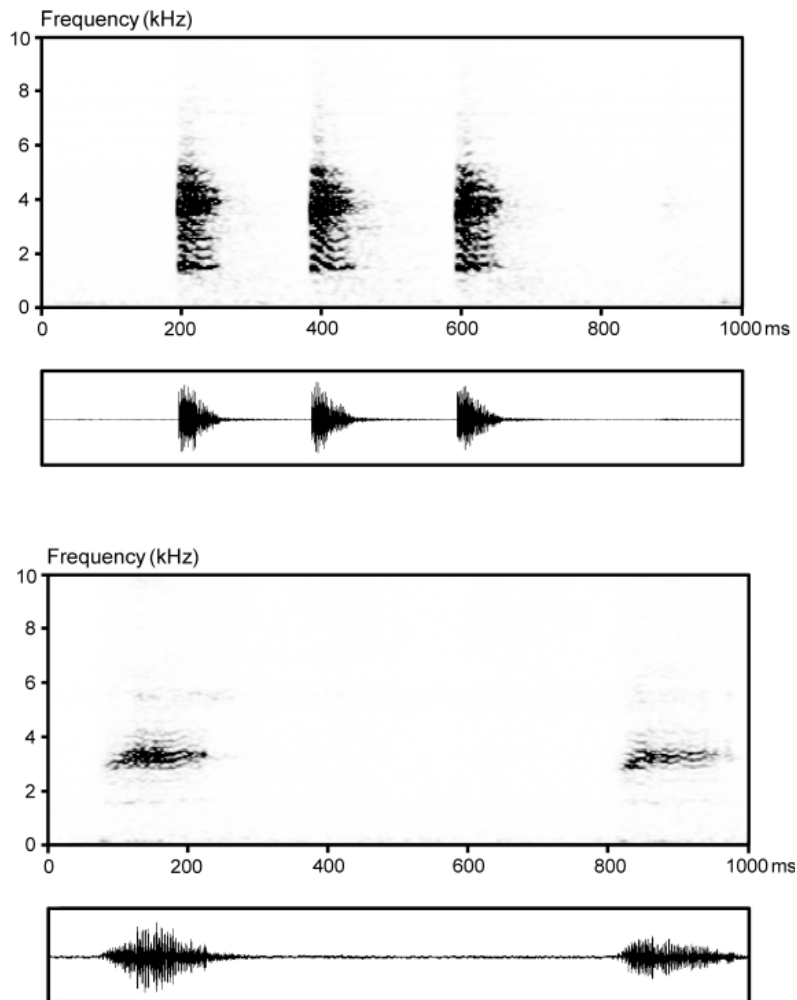


Fig. 1. Sonograms and oscillograms of calls of *Mantidactylus zavana* from Antsahamanara (above; group of three notes) and of *M. granulatus* from Andampy (below; two notes of a longer note series). Both localities are in the Manarikoba Forest in the Tsaratanana Massif.

Molecular phylogeography of *M. granulatus*

All three maximum parsimony analyses (coding gaps as fifth character or as uncertainties, and excluding sites with gaps), and the neighbour-joining analysis treating gaps as missing data agreed in the recovered phylogenetic topology (Fig. 2). A deep split between two groups was noted: one lineage contained the two specimens from Antsahamanara and Manarikoba and corresponded to the populations with divergent calls; the second lineage contained all other specimens. Maximum bootstrap support (100 %) was found for the monophyly of these two clades. In the second lineage, a further split was resolved: one lineage contained the specimens from Andampy

and Andapa, and the other lineage the specimens from Nosy Be and Montagne d'Ambre (bootstrap support 76–93 %). Identical results were obtained using the complete sequences and coding lacking sections as missing data.

In the restricted fragment, pairwise divergences (not considering indels) were 23–27 nt between the two major clades, 4–8 nt between the secondary clades, and 2–3 between sister specimens. In the complete fragment (557 nt), divergence between one sequence of each of the two major clades was 48 nt (8.6 %), and divergence between conspecific specimens of *M. granulatus* was 4–8 nt (as in the reduced fragment), corresponding to 0.7–1.4 %.



Fig. 2. Maximum parsimony (MP) phylogram based on 287 nucleotides of the mitochondrial 16S rRNA gene in four specimens of *Mantidactylus granulatus* and two specimens of *M. zavona*. The analysis was performed coding gaps as fifth character state. The numbers are bootstrap values (2000 replicates) in percent for MP coding gaps as fifth state (left), MP after exclusion of the three sites with gaps (centre), and neighbour-joining which treats gaps as missing characters (using LogDet distances; right). *M. leucomaculatus* was used as the outgroup. Bars mark apomorphies in sequence evolution within the *granulatus/zavona* lineage as reconstructed by PAUP*; thin grey bars are transitions, thin white bars are insertions, thick black bars are transversions. Biogeographic regions (Angel 1942; Glaw & Vences 1994) are abbreviated as follows: NE, Northeast; N, North; NC, Northern Central; NW, Northwest.

The high divergences of the major clades correlated with important differences in advertisement calls, even in conditions of sympatry (air distance between the localities Andampy and Antsahammanara is only about 2 km), and are therefore clearly indicative of separation at the species level. Specimens from the Manarikoba Forest differ from those from Nosy Be, the type locality of *M. granulatus*; we therefore describe them as new species in the following.

***Mantidactylus zavona* n.sp., Figs 3, 4, 5**

Diagnosis. A species of the genus *Mantidactylus* based on the absence of nuptial pads and presence of femoral glands in males. Attributed to the *Mantidactylus granulatus* group in the subgenus *Phyllacomantis* based on (a) moderate body size (SVL 35–44 mm), (b) laterally blackish, paired subgular vocal sacs, (c) lateral metatarsalia separated by webbing, (d) patch-like femoral glands of type 2 *sensu* Glaw *et al.* (2000), (e) calling from positions along brooks, and (f) high phenetic similarity to *M. granulatus*. Within the *M. granulatus* group, distinguished from *M. cornutus*, *M. redimitus*, *M. tandroka* and *M. tschenki* by the absence of interocular tubercles and from *M. leucomaculatus* by a larger inner metatarsal tubercle in males. By molecular analyses (Vences & Glaw 2001) it was grouped as sister species of *M. granulatus*, which is also morphologically most similar. *M. zavona*

differs from *M. granulatus*, however, by a smaller body size (male SVL 35–41 mm vs 40–45 mm), a shorter and wider head, larger femoral glands (see morphological comparison below), and advertisement calls (groups of three notes rather than single notes).

Holotype. ZSM 648/2001, adult male, collected by M. Vences, F. Andreone, F. Mattioli & J. Randrianirina on 2 February 2001 at Antsahammanara (14°02'42"S, 48°47'04"E; c. 1100 m above sea level) in the Manarikoba Forest, Réserve Naturelle Intégrale de Tsaratanana, central northern Madagascar.

Paratypes. ZSM 647/2001 and 649–650/2001, MRSN A2054 (FAZC 11003), A2056 (FAZC 11015), A2057 (FAZC 11024), A2058 (FAZC A11025), A2059 (FAZC 11051), A2060 (11119) and A2061 (FAZC 11168), 10 adult males, MRSN A2055 (FAZC 11005), A2062 (FAZC 11087), A2063 (FAZC 11088), three adult females, MRSN A2064 (FAZC 11089) and A2065 (FAZC 11142), two subadults, PBZT 11022, 11023, 11026, 11052, 11053, 11054, 1118, 11120, 11148, nine adult males, all collected by M. Vences, F. Andreone, F. Mattioli & J. Randrianirina between 2 and 13 February 2001 at the type locality, and three additional specimens of unknown sex with same collecting data: UADBA-MV 2001.57, 2001.85 and 2001.89. MSNG 49128 and UADBA 10020, two adult males, collected in the Manarikoba Forest at 14°02'24"S, 48°47'02"E by



Fig. 3. Holotype (ZSM 648/2001) of *Mantidactylus zavona*.

R. Jesu, G. Schimmenti and J. C. Piso, on 15–22 February 1997.

Description of the holotype. Specimen in excellent state of preservation. Part of right shank taken as tissue sample for genetic analysis. SVL 35.6 mm. For measurements see Table 2. Body moderately stout; head longer than wide, not wider than body; snout slightly pointed in dorsal view, rounded in lateral view; nostrils directed posterolaterally, slightly protuberant, much closer to tip of snout than to eye; canthus rostralis distinct, straight; loreal region concave; tympanum distinct, elliptical (slightly higher than wide), 54 % of horizontal eye diameter; supratympanic fold distinct, straight; tongue ovoid, distinctly bifid posteriorly; vomerine teeth distinct, in two rounded aggregations, positioned posterolateral to choanae; choanae rounded. Arms slender, sub-articular tubercles single; state of metacarpal tubercles not clearly discernible; fingers without webbing; relative length of fingers $1 < 2 < 4 < 3$, second finger distinctly shorter than fourth finger; finger disks distinctly enlarged; nuptial pads absent. Hind limbs slender; tibiotarsal articulation reaches nostril; lateral metatarsalia largely separated by webbing; inner metatarsal tubercle distinct and large, outer metatarsal tubercle small; webbing formula between toes 1 (1), 2i (1.5), 2e

(0.75), 3i (2), 3e (1), 4i (2), 4e (1.75), 5 (0.5); relative toe length $1 < 2 < 3 < 5 < 4$. Skin on the dorsal surface smooth to slightly granular; back with indistinct longitudinal folds: one pair running from behind the eyes onto the anterior back; laterally from these, a pair of dorsolateral folds runs from c. 4 mm behind the supratympanic fold to the inguinal region. No supraocular spines or tubercles; no distinct enlarged tubercles in the cloacal region; ventral skin slightly granular on belly, smooth on throat where the presence of blackish, paired subgular vocal sacs are clearly discernible. Femoral glands poorly delimited and indistinct in external view.

Colouration in preservative dorsally dark brown, with some indistinct light mottling. Limbs brown with dark crossbands: four bands on forelimb, five on femur, five on tibia, three to four on foot and tarsus. Flanks brown with relatively sharp border to the pale ventral colouration. Head laterally brown, with an indistinct white line along the canthus rostralis; no frenal stripe. Throat light brown with a cream-coloured median stripe. Part of the chest (region of shoulder girdle) without dark pigment. Posterior to this region dark marbling is present. Posterior belly cream without dark markings. In life, the colouration was similar to that in preservative. The iris was light brown in



Fig. 4. Holotype (ZSM 648/2001) of *Mantidactylus zavona*, ventral view.

its upper half and dark brown in its lower half. The belly was whitish-beige. The femoral glands had a paler colour than the surrounding skin.

Variation. Morphologically, the paratypes agree well with the holotype. A single individual (subadult MRSN-FAZC 11142) has a white frenal stripe. Other specimens (ZSM 647/2001) have a paler colouration (grey-brown) than the holotype, with dark brown tympanic region and head side. Some individuals have an indistinct (MRSN-FAZC 11119) or distinct (MRSN-FAZC 11025) W-shaped marking on the anterior back (similar to *M. leucomaculatus*). MRSN-FAZC 11025 also resembles that species in having a reddish brown back with sharp dorsolateral border to the dark brown flanks and two dark brown spots between the eyes (but no interocular tubercles as in species of the *M. redimitus* group). In MRSN-FAZC 11051, the femoral gland in internal view is of type 2 (Glaw *et al.* 2000) and is formed by 24 granules of 0.4–0.5 mm in diameter.

Male SVL was 35.0–41.3 mm (37.8 ± 2.0 mm, $n = 11$), female SVL was 40.5–44.0 mm (42.2 ± 1.8 mm, $n = 3$); mean male SVL was 90 % of mean female SVL. Significant intersexual differences (Mann-Whitney U-test; $P < 0.05$) were found in SVL, relative tympanum size (ratio TD/SVL), and relative size of the inner metatarsal tubercle (ratios

IMTL/SVL and IMTW/SVL): males were smaller, had a relatively larger tympanum size and larger inner metatarsal tubercles.

Etymology. The specific epithet is derived from the Malagasy word (northern dialect) *zavona* (pronounce: zav-oana), meaning cloud. It refers to the prevailing conditions at its type locality where, during our stay, sunshine was only observed in the early morning while clouds and rain dominated the rest of the day. The name is used as a noun in apposition to the generic name.

Natural history. Calling males were abundant at the type locality, in primary rainforest, at dawn and during the first hours of the night. They were calling from positions 1–2 m in shrubs along a brook (up to 20 m from the water). For a description of advertisement calls, see above (all data referring to recordings of the holotype; Fig. 1). During the day, specimens were occasionally found in leaf litter. The female MRSN-FAZC 11005 contained 14 yellowish oocytes of different sizes (diameter up to 3.6 mm) as ascertained by dissection.

Morphological comparison with *M. granulatus*. One typical feature in *M. granulatus* is a frenal stripe, i. e. the presence of well-delimited white colour along the upper lip (Glaw & Vences 1994). However, this character is absent in some speci-

Table 2. Morphometric measurements (all in mm) of specimens of *Mantidactylus zavona* and *M. granulatus*. For abbreviations, see Materials and Methods; additional abbreviations: HT (holotype), LT (lectotype), PT (paratype), M (male), F (female), FGL, FGW (length and width of femoral gland). RHL (relative hind-limb length) gives the position reached by the tibiotarsal articulation when the hind limb is adpressed along the body; it is coded as follows: when adpressed along the body, the tibiotarsal articulation reaches (0) between eye and nostril; (1) the nostril; (2) the snout tip; (3) beyond the snout tip; or (4) widely beyond the snout tip.

| Catalogue number | Locality | Sex | SVL | HW | HL | TD | ED | END | NSD | NND | HAL | FORL | HIL | FOTL | FOL | IMTL | IMTW | FGL | FGW | RHL |
|--|------------------|-----|------|------|------|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|-----|-----|-----|
| <i>Mantidactylus zavona</i> | | | | | | | | | | | | | | | | | | | | |
| MRSN-FAZC 11003 (PT) | Antsahamanara | M | 39.8 | 13.6 | 14.2 | 2.8 | 4.4 | 4.0 | 1.9 | 3.8 | 10.8 | 22.3 | 64.3 | 29.2 | 19.8 | 1.7 | 1.4 | 6.1 | 1.7 | 1 |
| MRSN-FAZC 11015 (PT) | Antsahamanara | M | 41.3 | 12.7 | 14.6 | 2.9 | 4.8 | 3.6 | 2.4 | 3.9 | 11.7 | 23.8 | 67.9 | 31.1 | 21.3 | 2.4 | 1.8 | 4.5 | 1.1 | 0 |
| MRSN-FAZC 11024 (PT) | Antsahamanara | M | 36.6 | 12.9 | 13.8 | 2.6 | 4.5 | 3.8 | 2.0 | 3.8 | 11.5 | 24.3 | 70.2 | 31.1 | 21.2 | 2.0 | 1.5 | – | – | 3 |
| MRSN-FAZC 11025 (PT) | Antsahamanara | M | 39.5 | 13.2 | 13.8 | 2.7 | 4.5 | 3.5 | 1.8 | 3.7 | 12.9 | 25.5 | 70.8 | 32.2 | 22.7 | 2.2 | 1.5 | – | – | 2 |
| MRSN-FAZC 11051 (PT) | Antsahamanara | M | 38.8 | 13.0 | 13.6 | 2.6 | 4.0 | 3.3 | 2.0 | 3.4 | 10.7 | 22.8 | 64.5 | 28.3 | 19.9 | 2.2 | 1.5 | 4.6 | 1.5 | 1 |
| MRSN-FAZC 11119 (PT) | Antsahamanara | M | 35.7 | 12.5 | 12.9 | 2.6 | 4.1 | 3.4 | 1.8 | 3.6 | 10.4 | 21.4 | 60.6 | 26.7 | 18.2 | 1.9 | 1.4 | – | – | 1 |
| MRSN-FAZC 11168 (PT) | Antsahamanara | M | 37.9 | 13.4 | 13.8 | 2.7 | 3.9 | 3.8 | 2.0 | 3.7 | 11.5 | 23.7 | 63.8 | 28.7 | 20.1 | 2.1 | 1.5 | – | – | 2 |
| ZSM 647/2001 (PT) | Antsahamanara | M | 38.6 | 13.6 | 14.6 | 2.7 | 5.0 | 3.6 | 2.1 | 4.3 | 11.0 | 24.0 | 70.3 | 30.9 | 21.4 | 2.5 | 1.8 | 5.3 | 1.1 | 3 |
| ZSM 648/2001 (HT) | Antsahamanara | M | 35.6 | 12.6 | 13.6 | 2.5 | 4.6 | 3.5 | 1.8 | 3.4 | 10.1 | 21.3 | 60.6 | 26.8 | 18.2 | 2.1 | 1.7 | 4.2 | 1.3 | 1 |
| ZSM 649/2001 (PT) | Antsahamanara | M | 37.4 | 13.1 | 14.1 | 2.7 | 4.4 | 3.6 | 2.1 | 4.0 | 11.7 | 24.3 | 70.0 | 30.4 | 20.9 | 2.0 | 1.8 | 6.0 | 1.4 | 3 |
| ZSM 650/2001 (PT) | Antsahamanara | M | 35.0 | 13.0 | 13.7 | 2.4 | 4.4 | 3.6 | 2.0 | 3.8 | 10.7 | 23.4 | 64.9 | 28.8 | 20.4 | 2.0 | 1.6 | 4.7 | 1.5 | 3 |
| MRSN-FAZC 11005 (PT) | Antsahamanara | F | 40.5 | 14.2 | 15.1 | 2.6 | 4.7 | 3.7 | 2.4 | 4.3 | 11.5 | 25.4 | 71.5 | 31.8 | 21.6 | 1.7 | 0.8 | – | – | 1 |
| MRSN-FAZC 11087 (PT) | Antsahamanara | F | 42.2 | 14.6 | 16.2 | 2.9 | 4.4 | 3.9 | 2.4 | 4.2 | 11.9 | 24.9 | 76.3 | 33.7 | 22.7 | 1.5 | 0.8 | – | – | 2 |
| MRSN-FAZC 11088 (PT) | Antsahamanara | F | 44.0 | 15.1 | 15.7 | 2.9 | 5.1 | 3.9 | 2.4 | 4.4 | 12.8 | 26.9 | 78.9 | 34.6 | 23.3 | 1.3 | 0.9 | – | – | 3 |
| <i>Mantidactylus granulatus</i> | | | | | | | | | | | | | | | | | | | | |
| MRSN-FAZC 8011 | Nosy Be | M | 42.8 | 14.3 | 15.6 | 3.3 | 5.3 | 4.4 | 2.2 | 4.3 | 12.2 | 25.7 | 69.5 | 31.8 | 21.1 | 2.2 | 1.5 | 3.2 | 0.9 | 1 |
| MRSN-FAZC 8030 | Nosy Be | M | 44.5 | 14.5 | 16.3 | 3.2 | 5.0 | 4.6 | 2.2 | 3.8 | 12.8 | 28.4 | 72.4 | 32.5 | 22.5 | 2.1 | 1.4 | – | – | 0 |
| MRSN-FAZC 8032 | Nosy Be | M | 43.0 | 13.9 | 16.1 | 2.9 | 5.2 | 4.5 | 2.1 | 4.0 | 11.6 | 24.5 | 68.2 | 32.5 | 21.0 | 2.0 | 1.8 | 3.4 | 1.3 | 0 |
| MRSN-FAZC 8125 | Nosy Be | M | 41.5 | 13.2 | 15.7 | 2.8 | 4.9 | 4.4 | 2.2 | 3.9 | 11.3 | 24.3 | 70.4 | 30.3 | 20.9 | 2.2 | 1.7 | 4.4 | 1.7 | 2 |
| MRSN-FAZC 8148 | Nosy Be | M | 42.0 | 13.9 | 16.4 | 3.3 | 5.3 | 4.3 | 2.2 | 3.7 | 12.5 | 26.0 | 69.8 | 32.3 | 22.6 | 2.5 | 1.6 | – | – | 0 |
| MRSN-RJS 0063 | Andranomamelona | M | 42.0 | 14.2 | 16.3 | 3.0 | 5.0 | 4.6 | 2.3 | 4.0 | 12.6 | 26.4 | 75.0 | 33.7 | 23.2 | 2.2 | 1.6 | 4.5 | 1.5 | 2 |
| ZFMK 52708 | Andrakata | M | 39.7 | 12.8 | 14.5 | 2.9 | 4.5 | 4.1 | 2.5 | 3.5 | 12.3 | 24.9 | 70.3 | 32.0 | 22.1 | 1.8 | 1.4 | – | – | 3 |
| ZFMK 52709 | Andrakata | M | 41.5 | 12.9 | 15.0 | 3.0 | 4.2 | 4.5 | 2.4 | 3.4 | 12.4 | 26.0 | 76.1 | 34.2 | 23.5 | 2.3 | 1.6 | – | – | 3 |
| ZFMK 59961 | Andapa | M | 42.1 | 13.5 | 16.2 | 3.0 | 4.6 | 4.6 | 2.2 | 3.9 | 12.6 | 25.7 | 75.4 | 34.3 | 23.8 | 2.6 | 1.7 | – | – | 4 |
| ZFMK 59962 | Andapa | M | 42.7 | 13.6 | 16.1 | 3.4 | 4.4 | 4.8 | 2.3 | 3.6 | 13.0 | 27.0 | 78.4 | 35.0 | 24.1 | 2.3 | 1.6 | – | – | 4 |
| ZFMK 59963 | Andapa | M | 40.2 | 13.2 | 15.6 | 2.9 | 4.7 | 4.7 | 2.3 | 3.4 | 12.2 | 25.0 | 75.8 | 34.0 | 23.3 | 1.8 | 1.8 | 4.6 | 1.9 | 4 |
| ZSM 645/2001 | Andampy | M | 40.5 | 14.2 | 15.7 | 3.0 | 4.8 | 4.3 | 2.3 | 3.8 | 12.0 | 25.0 | 68.8 | 31.0 | 21.6 | 1.9 | 1.6 | – | – | 2 |
| ZSM 646/2001 | Andampy | M | 42.5 | 14.3 | 16.2 | 3.1 | 5.2 | 4.4 | 2.1 | 3.7 | 12.1 | 24.8 | 72.0 | 32.2 | 22.2 | 2.5 | 1.9 | – | – | 2 |
| BMNH 1987.2289 | Manongarivo | F | 42.9 | 13.7 | 16.6 | 2.7 | 5.1 | 4.2 | 2.6 | 3.7 | 12.5 | 25.8 | 82.0 | 36.1 | 24.0 | 1.5 | 0.8 | – | – | 3 |
| BMNH 1987.2290 | Manongarivo | F | 39.5 | 12.7 | 15.5 | 2.5 | 4.5 | 4.2 | 2.3 | 3.4 | 11.9 | 24.0 | 77.0 | 35.0 | 23.6 | 1.8 | 0.8 | – | – | 3 |
| MNHN 1893.238 | Montagne d'Ambre | F | 41.1 | 12.9 | 16.1 | 3.1 | 4.7 | 4.2 | 2.3 | 3.9 | 12.6 | 25.7 | 79.1 | 36.3 | 23.6 | 1.7 | 0.7 | – | – | 4 |
| MNHN 1973.913 | Marojejy | F | 39.9 | 12.7 | 16.0 | 2.9 | 4.8 | 4.1 | 2.1 | 3.3 | 11.6 | 25.3 | 74.2 | 33.2 | 22.8 | 1.4 | 0.8 | – | – | 4 |
| MNHN 1973.914 | Marojejy | F | 44.8 | 14.2 | 17.2 | 3.2 | 5.4 | 4.9 | 2.3 | 3.6 | 12.5 | 27.4 | 84.6 | 38.6 | 25.4 | 1.6 | 0.8 | – | – | 4 |
| MRSN-FAZC 8149 | Nosy Be | F | 44.9 | 14.5 | 17.8 | 3.3 | 5.6 | 4.4 | 2.4 | 4.2 | 12.6 | 26.1 | 75.5 | 34.2 | 22.7 | 2.0 | 1.3 | – | – | 1 |
| SMF 6728 (LT) | Nosy Be | F | 42.6 | 14.4 | 17.1 | 2.9 | 5.6 | 4.2 | 2.2 | 3.6 | 12.7 | 29.5 | 80.8 | 35.2 | 24.0 | 1.5 | 0.8 | – | – | 3 |

mens that otherwise clearly belong to this species. For instance, among the sample examined in our study (Table 2), a frenal stripe was present in all specimens from Manongarivo, Marojejy and Tsaratanana (Andampy and Andranomamelona), in one of three specimens from Andapa, and in two of three specimens from Andrakata. In the sample from the type locality Nosy Be, it was absent in four specimens, faint in one specimen and present in two specimens (among them the lectotype). However, according to the molecular data (Fig. 2), specimens without (Montagne d'Ambre) and with frenal stripe (Andampy, Andapa) showed only a low genetic differentiation and are considered to be conspecific. Although this character is variable in *M. granulatus*, the high frequency of its occurrence means that it is a valuable character to differentiate the species from *M. zavona*: in fact, of more than 20 specimens of *M. zavona*, only one subadult had a frenal stripe, indicating that it is a rare state in this species.

In the measured sample of *M. granulatus* (Table 2), male SVL was 39.7–44.5 mm (41.9 ± 1.3 mm, $n = 8$), female SVL was 39.5–44.9 mm (42.2 ± 2.2 mm, $n = 6$). Mean male SVL was 99 % of mean female SVL. Significant sexual dimorphism (Mann-Whitney U-test; $P < 0.001$) was found in the size of the inner metatarsal tubercle (ratios IMTL/SVL and IMTH/SVL; larger in males) but not in relative tympanum size or SVL.

In a comparison of male specimens of *M. zavona* and *M. granulatus* with Mann-Whitney U-tests, highly significant differences ($P < 0.001$) were found in SVL (larger in *M. granulatus*), and in a ratio of head measurements, HW/HL, reflecting the broader and shorter head shape of *M. zavona*. Although femoral glands could be reliably measured in only five *M. granulatus* and seven *M. zavona*, differences in FGL/SVL also tested significant ($P < 0.01$; shorter in *M. granulatus*). In one male of *M. granulatus* (MRSN-FAZC 8011), the femoral gland (in internal view) was of type 2 (Glaw *et al.* 1998) and was formed by 15 granules of up to 0.5 mm in diameter. Femoral glands in *M. granulatus* are thus usually smaller, composed of fewer granules and less distinct than in *M. zavona*. In females, sample size was smaller (Table 2), and significant interspecific differences were only detected in the ratio HW/HL ($P < 0.05$). The lectotype of *M. granulatus* (SMF 6728) corresponded in morphology to other species attributed to the species (Table 2).

Distribution of *M. granulatus* and *M. zavona*

Considering data summarized here (Fig. 5), the new species *M. zavona* is so far known only from two sites in the Manarikoba Forest (Tsaratanana Massif), namely the type locality Antsahamanara and a second site represented by the MSNG vouchers. It is therefore known only from higher altitudes around 1000 m above sea level.

By contrast, *M. granulatus* is mainly known from low- to mid-altitude sites (see appendix for voucher specimens): (1) Nosy Be (type locality), (2) Benavony, (3) Manongarivo, (4) Andampy (Tsaratanana), (5) Andranomamelona (Tsaratanana), (6) Montagne d'Ambre, (7) Marojejy, (8) Ambatomitatao (Marojejy, 400 m; Glaw & Vences 1994); (9) Andapa, (10) Andrakata (Fig. 5). Known altitudinal range is from a few metres above sea level (Nosy Be) to 850 m (Andranomamelona), and probably up to 1250 m at Montagne d'Ambre (Raxworthy & Nussbaum 1994; vouchers not examined by us). Blommers-Schlösser & Blanc (1991) listed three additional localities for the species: Antsingy, Behara and Amboasary. While we could not find any voucher specimen for Antsingy in the MNHN and ZMA collections on which the distribution records of these authors are almost exclusively based, two specimens originate from 'Behara, region d'Amboasary': MNHN 1975.495–496.

Both specimens are in poor state of preservation. Colour and pattern are completely faded. MNHN 1975.495 measures 20.4 mm SVL and probably belongs to a species in the *M. domerguei* group of the subgenus *Blommersia*. The second specimen is an adult male with well-developed femoral glands that measures 29.7 mm SVL. Its taxonomic identity cannot be ascertained, but the small size excludes the possibility of it belonging to *M. granulatus*.

DISCUSSION

Our data revealed a limited overall genetic differentiation among populations of *Mantidactylus granulatus* from the different biogeographic regions in northern Madagascar. Although some bioacoustic and morphological divergence of specimens from the north-east was evident (e.g., longer note duration in specimens from Andrakata and Andapa), the genetic divergence was low in the studied sequences. Assuming that the different haplotypes identified within *M. granulatus* correspond to a geographic subdivision

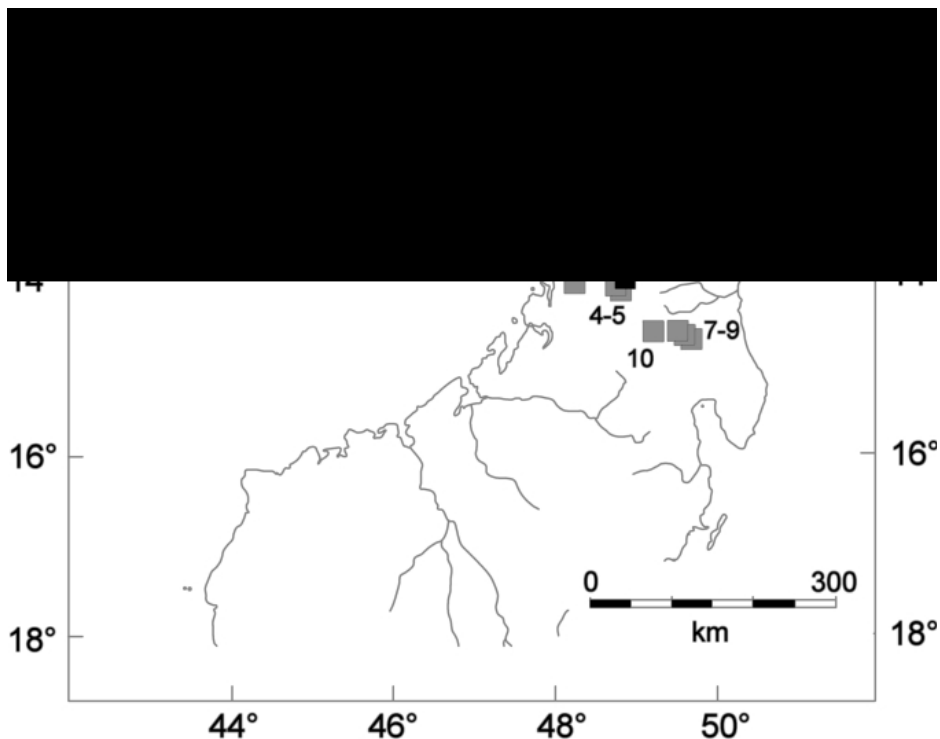


Fig. 5. Distribution of *Mantidactylus zavona* and *M. granulatus* in northern Madagascar. Numbers of localities correspond to those used in the text.

of the species and do not represent haplotype polymorphism within populations (Avice 2000), it can be concluded that gene flow among *M. granulatus* populations of the whole of northern Madagascar has been possible in relatively recent times, probably in the Pliocene–Pleistocene if usual calibrations are applied (Avice 2000). As *M. granulatus* is largely restricted to areas with at least rudimentary forests, our data therefore indicate a recent connection of the lowland forest blocks of northern Madagascar, including the now largely isolated Montagne d'Ambre Massif.

The Tsaratanana Massif harbours three endemic nominal frog species according to Blommers-Schlösser & Blanc (1991), all belonging to the subfamily Cophylinae in the family Microhylidae: *Plethodontohyla guentherpetersi*, *Platypelis alticola* and *Platypelis tsaratananaensis*. While no elevational information is available for the holotype and only specimen of *P. alticola*, the two other species were collected from high altitudes only (2600 m). During our survey in the Manarikoba Forest on the Tsaratanana Massif, we found only one potentially endemic, probably undescribed frog of the taxonomically unrevised subgenus

Brygroomantis at Andampy. All other mantellid species collected at this mid-altitude site were known from either Montagne d'Ambre (*Mantidactylus ambreensis*, *Boophis blommersae*), Marojejy (*Mantidactylus pseudoasper*, *M. granulatus*) or Benavony (*Boophis brachychir*, *M. granulatus*, *M. pseudoasper*), or from other sites (F. Andreone, F. Mattioli, J. Randrianirina and M. Vences, unpubl. data). At the higher site Antsahamanara (1000 m elevation), at least one species (*Mantidactylus zavona*) was a probable Tsaratanana endemic, while several others (*Mantidactylus* cf. *peraccae*, *M. cf. fimbriatus*) were further candidates. Two microhylids, belonging to the genera *Platypelis* and *Plethodontohyla*, apparently were undescribed species, but had already been found by us in further surveys in low- and mid-altitude NE sites (Andreone *et al.*, 2000; Andreone & Randrianirina, unpubl.). The highest percentage of endemics is probably found among the montane fauna; of the eight frog species listed by Raxworthy & Nussbaum (1996) as montane specialists of Tsaratanana, all were endemics of this massif. Hence, Tsaratanana appears to harbour an amphibian fauna characterized by increasing endemism at

higher altitudes, but more taxonomic work is necessary before reliable percentages of endemism for the different elevational levels can be calculated. A similar situation may apply to the Marojejy Massif in which at least one species, *Mantidactylus schilfi*, appears to be endemic to high altitudes (Glaw & Vences 2001).

These biogeographic patterns may indicate a successively older isolation of the corresponding habitats in the course of climatic oscillations: while low altitude forests with frogs such as *M. granulatus*, were in contact until rather recently, the mid- and high-altitude forests were isolated for longer times – allowing speciation events that gave rise to regional endemics such as *M. zavona*.

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Appendix 1. Comparative material examined.

Mantidactylus granulatus. (a) males: MRSN-FAZC 8011, 8030, 8032, 8125, 8148 (Nosy Be); MRSN-RJS 0063 (Andranomamelona, Tsaratanana, 850 m altitude); UADBA-MV 2001.152 and ZSM 645–646/2001 (Andampy, Manarikoba forest, Tsaratanana, 730 m altitude); ZFMK 59961–59963 (Andapa); ZFMK 52708–52710 (Andrakata). (b) females: MNHN 1973.913–914 (Marojejy, 300 m altitude); BMNH 1987.2289–2290 (Manongarivo, 350 m altitude); MNHN 1893.238 (Montagne d'Ambre); MRSN-FAZC 8149 (Nosy Be); SMF 6728 (lectotype, Nosy Be).

Mantidactylus leucomaculatus. – (a) males: MNHN 1973.917 (Marojejy, 300 m altitude); MNHN 1975.512–514 (Marojejy); MRSN-FAZC 10326 (Ilampy, 470 m altitude); MRSN-FAZC uncatalogued, two males (Ambanizana, <20 m altitude); ZFMK 52706 (Nosy Boraha, <200 m altitude); ZFMK 52707 (Nosy Mangabe, <200 m altitude); ZFMK 59934–59935 and 59953–59954 (Marojejy, Camp 1, 300 m altitude). (b) females: MNHN 1973.915–916, 1973.918 (Marojejy, 300 m altitude); MNHN 1975.1 (holotype; Nosy Mangabe); MRSN A1885 and A1851 (Tsararano, 700 m altitude); ZFMK 59936 (Marojejy, Camp 1, 300 m altitude).